PROJECT NUMBER: 6906
PROJECT TITLE: Biological Effects of Smoke
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I. INHIBITION OF EGF ASSAY

A. **Objective**: Establish the EGF assay and determine the effects of various CSCs and related products on EGF binding.

B. **Results**: A second experiment was conducted to evaluate the effects of the acid, base, and neutral fractions obtained from IT 2R1 CSC and IT 100% ES CSC at a final concentration of 150 ug/ml. As in the previous experiment, each fraction reduced cell number and inhibited EGF binding. The relative rank order of both CSC samples tested for the inhibition of EGF binding were unfractionated CSC > base > acid > neutral. These results (rank order) were similar to those observed in the previous experiment.

C. **Plans**: No additional experiments are planned regarding the activity of fractions obtained from the CSCs mentioned above.

D. **References**:

Patskan, G. J. Notebook No. 8674, p. 93.

II. GLUTATHIONE DEPLETION ASSAY (GDA)

A. **Objective**: To determine the effect of the reduction in cellular GSH by 2R1 CSC on *Salmonella* TA98 activity of the direct acting control compound, 2-nitrofluorene (2NF).

B. **Results**: With a 2R1 CSC pretreatment dose of 0.50 mg/plate, the cellular GSH level was reduced and no significant increase in 2NF activity (revertants/plate) was observed in these samples. This suggests that the reduction in GSH levels by 2R1 CSC does not affect the activity of 2NF. However, the results from this experiment were compromised by the apparent CSC toxicity. Another experiment using a lower dose of 2R1 CSC (0.25 mg/plate) was successful in eliminating 2R1 CSC toxicity as well as producing a reduction in the GSH level. However, no significant difference in 2NF activity was observed as a result of the CSC-induced reduction of GSH levels. One possible explanation for this observation could be that the GSH levels returned to control levels following CSC pretreatment and *before* the cells were affected by 2NF.

C. **Plans**: To pretreat cells with 2R1 CSC and 2NF at the same time.

D. **References**:

McCoy, W. R. Notebook No. 7127, p. 188.

III. PROTEIN KINASE C (PKC) WHOLE CELL ASSAY

A. **Objective**: To determine the optimum cell conditions, incubation time and incubation medium for treating 3T3 cells in the PKC whole cell assay.

B. **Results**: Three cell conditions were tested: quiescent, high glucose quiescent, and log phase. The incubation solutions tested were standard DMEM media and whole cell buffer (WCB) which contains neither serum nor phosphate. Samples were incubated with 200 ug fresh 2R1 CSC per ml for 15 min., 5 hr., or 19.5 hr. Results indicated that cells require incubation in standard media while being exposed to CSC for extended periods of time. Log phase cells show increased levels of phosphorylation after exposure to CSC.

C. **Plans**: Repeat the different incubation times of log phase cells with CSC. Test several compounds for use as positive controls. Test stored CSC.

D. **References**:

Nixon, G.M. Notebook No. 8569, p. 179.